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The Pentose Phosphate Pathway of Glucose Metabolism

HORMONAL AND DIETARY CONTROL OF THE OXIDATIVE AND NON-OXIDATIVE REACTIONS OF THE CYCLE IN LIVER

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1. Measurements were made of the non-oxidative reactions of the pentose phosphate cycle in liver (transketolase, transaldolase, ribulose 5-phosphate epimerase and ribose 5-phosphate isomerase activities) in a variety of hormonal and nutritional conditions. In addition, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities were measured for comparison with the oxidative reactions of the cycle; hexokinase, glucokinase and phosphoglucose isomerase activities were also included. Starvation for 2 days caused significant lowering of activity of all the enzymes of the pentose phosphate cycle based on activity in the whole liver. Re-feeding with a high-carbohydrate diet restored all the enzyme activities to the range of the control values with the exception of that of glucose 6-phosphate dehydrogenase, which showed the well-known 'overshoot' effect. Re-feeding with a high-fat diet also restored the activities of all the enzymes of the pentose phosphate cycle and of hexokinase; glucokinase activity alone remained unchanged. Expressed as units/g. of liver or units/mg. of protein hexokinase, glucose 6-phosphate dehydrogenase, transketolase and pentose phosphate isomerase activities were unchanged by starvation; both 6-phosphogluconate dehydrogenase and ribulose 5-phosphate epimerase activities decreased faster than the liver weight or protein content. 2. Alloxan-diabetes resulted in a decrease of approx. 30–40% in the activities of 6-phosphogluconate dehydrogenase, ribose 5-phosphate isomerase, ribulose 5-phosphate epimerase and transketolase; in contrast with this glucose 6-phosphate dehydrogenase, transaldolase and phosphoglucose isomerase activities were unchanged. Treatment of alloxan-diabetic rats with protamine–zinc–insulin for 3 days caused a very marked increase to above normal levels of activity in all the enzymes of the pentose phosphate pathway except ribulose 5-phosphate epimerase, which was restored to the control value. Hexokinase activity was also raised by this treatment. After 7 days treatment of alloxan-diabetic rats with protamine–zinc–insulin the enzyme activities returned towards the control values. 3. In adrenalectomized rats the two most important changes were the rise in hexokinase activity and the fall in transketolase activity; in addition, ribulose 5-phosphate epimerase activity was also decreased. These effects were reversed by cortisone treatment. In addition, in cortisone-treated adrenalectomized rats glucokinase activity was significantly lower than the control value. 4. In thyroidectomized rats both ribose 5-phosphate isomerase and transketolase activities were decreased; in contrast with this transaldolase activity did not change significantly. Hypophysectomy caused a 50% fall in transketolase activity that was partially reversed by treatment with thyroxine and almost fully reversed by treatment with growth hormone for 8 days. 5. The results are discussed in relation to the hormonal control of the non-oxidative reactions of the pentose phosphate cycle, the marked changes in transketolase activity being particularly outstanding.

Although there have been many studies of changes in liver glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities in different hormonal conditions and in various nutritional states there have been only relatively few assays under optimum conditions of enzymes

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of the non-oxidative part of this cycle (Hollman, 1964; Horecker, 1964; Benevenga, Stielau & Freedland, 1964; Srivastava & Hübscher, 1966; Dreyfus, 1967; Novello & McLean, 1968). Insulin, thyroxine, oestrogens, adrenal steroids and pituitary hormones have all been shown to have a marked effect in the control of the dehydrogenases of the pentose phosphate cycle in liver (Glock & McLean, 1955; Huggins & Yao, 1959; Dickens, Glock & McLean, 1959; Fitch, Hill & Chaikoff, 1959; Willmer, 1960; Weber & McDonald, 1961; Tepperman & Tepperman, 1962; Weber, 1963). The question arises whether these effects are accompanied by parallel changes in the activities of the non-oxidative enzymes of the cycle.

It has been shown that the activities of rat liver transketolase and transaldolase measured under optimum conditions are closely similar to each other and to that of glucose 6-phosphate dehydrogenase, whereas ribose 5-phosphate isomerase and ribulose 5-phosphate 3-epimerase activities were more than twofold and tenfold greater respectively than that of transketolase and were therefore not likely to be rate-limiting in this cycle. There is the possibility either that these enzymes may behave as a constant-proportion group (Pette, Klingenberg & Bücher, 1962; Pette, Luh & Bücher, 1962), parallel changes being found in all enzymes of the cycle, or that a particular rate-limiting enzyme of the non-oxidative part of the cycle may be more susceptible to control by hormonal and dietary factors.

In the present work the hormonal conditions studied were alloxan-diabetes and the effect of insulin treatment, hypothyroidism, hypophysectomy followed by replacement therapy with thyroxine and growth hormone, glucagon administration and the effect of adrenal steroids as shown in adrenalectomized rats and cortisone-treated animals. In addition, the effects of starvation followed by re-feeding with a diet high in carbohydrate or fat on the enzymes of the pentose phosphate cycle were studied. To present a complete picture of the whole pathway and to make direct comparisons with changes found in the oxidative part of the cycle assays of both glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities were included. Hexokinase, glucokinase and phosphoglucose isomerase activities were also measured since these enzymes may be considered an integral part of the whole cycle, phosphoglucose isomerase being involved in the complete recycling of glucose 6-phosphate in the pentose phosphate cycle converting fructose 6-phosphate produced from the transaldolase and transketolase reactions into glucose 6-phosphate.

METHODS

Materials. $NADP^+$, NAD^+, NADH, ATP (sodium salt), glucose 6-phosphate (sodium salt), ribose 5-phosphate (barium salt) and fructose 6-phosphate (barium salt) were obtained from Boehringer Corp., London, W. 5. The barium salts of the sugar phosphates were converted into their potassium salts by treatment with K_2CO_3; the excess of carbonate was removed by treatment with HCl and the solutions were adjusted to pH7·2. Glucose 6-phosphate dehydrogenase (140units/mg.), glycerol 1-phosphate dehydrogenase–triose phosphate isomerase mixture (the relative activities of the two components being 1:6) (10mg./ml.) and phosphoglucose isomerase (390units/mg.) were obtained from Boehringer Corp. L-Thyroxine was purchased from Sigma Chemical Co. (St Louis, Mo., U.S.A.). Growth hormone (bovine) was a gift from the Endocrinology study section of the National Institutes of Health, Bethesda, Md., U.S.A. All other reagents were standard commercial products with the exception of the following sugar phosphates and enzyme preparations required in the assay of the non-oxidative reactions of the pentose phosphate cycle: erythrose 4-phosphate, ribose 5-phosphate free from pentulose, an equilibrium mixture of ribulose 5-phosphate and xylulose 5-phosphate, partially purified transketolase free from ribose 5-phosphate isomerase and ribulose 5-phosphate epimerase, a preparation of a mixture of ribose 5-phosphate isomerase and ribulose 5-phosphate epimerase, and a preparation of ribose 5-phosphate isomerase free from ribulose 5-phosphate epimerase and transketolase; these were prepared as described by Novello & McLean (1968).

Animals and hormonal and dietary treatment. (*a*) Starvation and re-feeding with high-carbohydrate and high-fat diets. Adult male albino rats (initial body wt.170–190g.) were used and 26 animals were divided into four groups: a control group of eight animals received *ad lib.* the standard laboratory rat cube M.R.C. diet 41B containing 65% carbohydrate, 16% protein and 3% fat; the remaining animals were starved for 48hr.; six were re-fed with a diet high in carbohydrate (white bread, 83% carbohydrate, 14% protein, 3% fat), and six received a diet high in fat (60% fat, 1% carbohydrate, 30% protein, 9% cellulose filler) for periods of 72hr. and six animals were killed at the end of the starvation period.

(*b*) Alloxan-diabetes and treatment with insulin. Adult male albino rats (initial body wt. approx. 220–250g.) were starved for 48hr. and then alloxan monohydrate was injected subcutaneously in a single dose of 20mg. of alloxan/100g. body wt. The alloxan-diabetic rats were then given 2 units of protamine–zinc–insulin daily for the first 5 days after administration of alloxan; this greatly increased the overall survival rate. Thereafter the insulin was withdrawn and the rats were maintained on the stock diet *ad lib.* for 3 weeks. After this period the alloxan-diabetic rats were divided into three groups: one group was maintained without further treatment, the second group received daily injections of protamine–zinc–insulin (2 units) for 3 days and the third group was treated with 2 units of protamine–zinc–insulin for 7 days.

(*c*) Glucagon treatment. Young male albino rats (initial body wt. approx. 160g.) were used. Three doses of glucagon, each of 100μg., were administered daily by subcutaneous injection. Control animals received injections of 0·9% NaCl.

(*d*) Adrenalectomy and treatment with cortisone. Young male albino rats (initial body wt. approx. 160–170g.) were adrenalectomized or submitted to a sham operation. The adrenalectomized rats were given 1% NaCl in the drinking water and the control rats were pair-fed with the adrenalectomized animals on the normal laboratory stock diet. These animals were used 1 week later. A second group of adrenalectomized rats were given three doses of cortisone (100μg. of cortisone hemisuccinate each dose) subcutaneously and killed 3 days later; again a paired-feeding regime was maintained with a separate group of control animals.

(*e*) Cortisone treatment alone. Adult male albino rats (initial body wt. approx. 170–190g.) were used. Cortisone hemisuccinate was given subcutaneously, 5mg. daily for 3 days, and the animals were killed on the fourth day.

(*f*) Thyroidectomy. Adult male albino rats (initial body wt. approx. 150–160g.) were thyroidectomized or submitted to a sham operation under ether anaesthesia. Since removal of the thyroid gland in rats also entails the removal of the parathyroids embedded in them, 1% (w/v) calcium gluconate was given in the drinking water to alleviate the effects of parathyroidectomy. Control rats were pair-fed with the thyroidectomized rats since there was a considerable decrease in food intake in the thyroidectomized rats. The animals were killed on the eleventh day after the operation.

(*g*) Hypophysectomy and treatment with thyroxine and growth hormone. Hypophysectomized and control male rats were purchased from the Charles River Co. (Wilmington, Mass., U.S.A.) and were fed on stock diet, supplied *ad lib.* and supplemented by 5% (w/v) glucose in the drinking water, until used 8 days after hypophysectomy. The rats were then divided into four groups: (1) normal control rats; (2) hypophysectomized rats; (3) hypophysectomized rats treated with 0·5mg. of growth hormone/day; (4) hypophysectomized rats treated with 10μg. of L-thyroxine/day; treatment was continued for 8 days, during which period the food intake of each group was matched, by limit-feeding, to the amount consumed by the hypophysectomized group.

Preparation of tissue homogenates. Liver homogenates were usually prepared in a medium containing 0·15M-KCl, 5mM-$MgCl_2$, 5mM-EDTA and 0·1mM-dithiothreitol adjusted to pH7·4 with $KHCO_3$ by using the proportions 1g. of liver to 9ml. of medium. The homogenate was centrifuged at 100000*g* for 40min. at 0° and the supernatant was taken and dialysed, with stirring, for 1–2hr. against the same extraction medium. With the groups of hypophysectomized rats 10mM-mercaptoethanol was used instead of dithiothreitol. The dialysed preparations were used for the determination of all the enzyme activities.

Assay of enzymes. Hexokinase (D-hexose–ATP phosphotransferase, EC 2.7.1.1), the enzyme with a low K_m for glucose, and glucokinase (D-glucose–ATP phosphotransferase, EC 2.7.1.2), the enzyme with a high K_m for glucose, were assayed essentially by the method of Sharma, Manjeshwar & Weinhouse (1963) with modifications as described by McLean & Brown (1966). A unit of enzyme is defined as the amount catalysing the formation of 1μmole of glucose 6-phosphate/min. at 25°. Glucose 6-phosphate dehydrogenase (D-glucose 6-phosphate–NADP oxidoreductase, EC 1.1.1.49) and 6-phosphogluconate dehydrogenase [6-phospho-D-gluconate–NADP oxidoreductase (decarboxylating), EC 1.1.1.44] were assayed by the method of Glock & McLean (1953). Ribulose 5-phosphate epimerase (D-ribulose 5-phosphate 3-epimerase, EC 5.1.3.1), ribose 5-phosphate isomerase (D-ribose 5-phosphate ketol-isomerase, EC 5.3.1.6), transketolase (sedoheptulose 7-phosphate–D-glyceraldehyde 3-phosphate glycolaldehydetransferase, EC 2.2.1.1) and transaldolase (sedoheptulose 7-phosphate–D-glyceraldehyde 3-phosphate dihydroxyacetonetransferase, EC 2.2.1.2) were assayed by the methods described by Novello & McLean (1968). Phosphoglucose isomerase (D-glucose 6-phosphate ketol-isomerase, EC 5.3.1.9) was assayed by the method of Noltmann (1966). The rate of reduction of $NADP^+$ or reoxidation of NADH were measured in a Unicam SP.800 recording spectrophotometer with a constant-temperature cell housing and scale-expansion accessory. In each case a unit of enzyme activity is defined as the amount catalysing the formation of 1μmole of product/min. at 25°.

Determination of protein. Protein was determined in the dialysed high-speed supernatant fractions by the method of Lowry, Rosebrough, Farr & Randall (1951).

Statistical treatment. Results are expressed as units of enzyme activity $\pm$S.E.M. The differences are regarded as being significant where Fisher's P is less than 0·05; where P is greater than 0·1 this is shown as 'not significant' (N.S.).

RESULTS

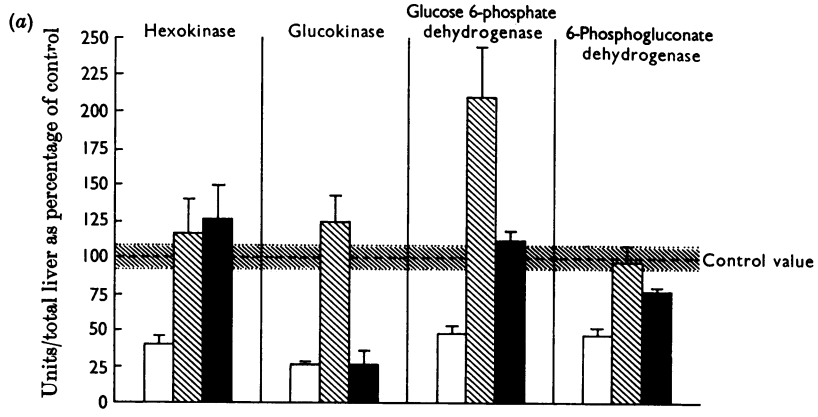
Effect of starvation and re-feeding with diets high in carbohydrate and fat. The results of the experiments in which rats were starved for 48hr. and re-fed for 3 days are given in Table 1 as enzyme activity/g. of liver and in Fig. 1 where the changes occurring in the total liver are shown. Fig. 1 shows that the total activity in liver of all enzymes of the pentose phosphate pathway and of phosphoglucose isomerase were decreased to about the same extent during starvation for 48hr.; in addition, hexokinase and glucokinase activities were greatly decreased. The fall in glucokinase activity was the most marked, a decline to less than 30% of the control value being found. However, because of the fall in liver weight and rise in the supernatant protein content the activities when expressed as units/g. of liver or units/mg. of protein were not all significantly decreased (see Table 1). Both 6-phosphogluconate dehydrogenase and ribulose 5-phosphate epimerase activities decreased faster than the liver weight; in contrast with this transketolase and transaldolase activities both increased slightly when expressed on this basis; again the most outstanding difference was in glucokinase activity. The total transketolase and transaldolase activities of the liver had fallen to $67 \pm 4{\cdot}4$% and $65 \pm 4{\cdot}5$% of the control values respectively after a 48hr. period of starvation, and no further decrease was found at 72hr., when the values as percentages of the activities found in control fed rats were $66 \pm 3{\cdot}8$% and $65 \pm 4{\cdot}2$% respectively.

The pattern of change on re-feeding with a high-carbohydrate diet is most clearly seen in Fig. 1. The outstanding changes are the well-known rise

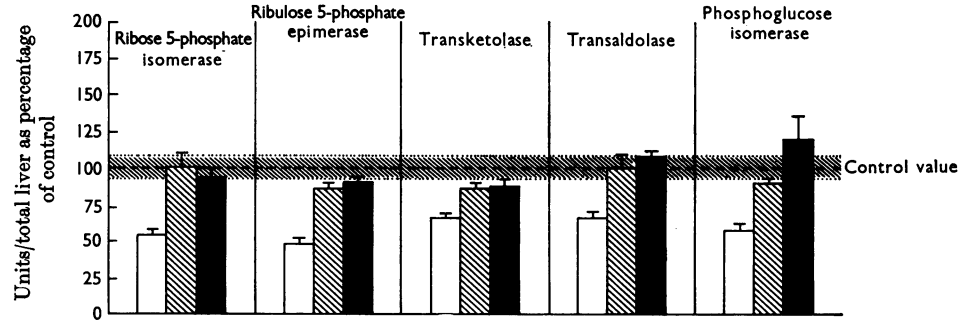
Table 1. *Effects of starvation and re-feeding with high-carbohydrate and high-fat diets on the activities of enzymes of the pentose pathway in rat liver*

A unit of enzyme activity is the amount catalysing the formation of 1 μmole of product/min. at 25°. The results are given as means $\pm$ S.E.M. Fisher's P values are given; where P is greater than 0·1 the value is quoted as N.S. (not significant). The experimental groups included rats starved for 48 hr. and then re-fed either with high-carbohydrate diet or high-fat diet for 3 days, as described in the Methods section.

Group no.	Control 1	Starvation (48 hr.) 2	Starvation (48 hr.), re-feeding with high-carbohydrate diet 3	Starvation (48 hr.), re-feeding with high-fat diet 4	Fisher's P for comparison of groups				
					1 versus 2	1 versus 3	1 versus 4	2 versus 3	2 versus 4
No. of animals	8	6	6	6					
Body wt. (g.)	183 $\pm$ 6	126 $\pm$ 5	129 $\pm$ 6	166 $\pm$ 3·4					
Liver wt. (g.)	8·46 $\pm$ 0·41	4·74 $\pm$ 0·35	6·71 $\pm$ 0·47	7·51 $\pm$ 0·25					
Protein content (mg./g.)	92·4 $\pm$ 3·0	110 $\pm$ 4·0	94·7 $\pm$ 5·0	89·0 $\pm$ 1·0					
Units/g. of liver									
Hexokinase	0·247 $\pm$ 0·049	0·178 $\pm$ 0·026	0·370 $\pm$ 0·085	0·364 $\pm$ 0·065	N.S.	N.S.	N.S.	< 0·05	< 0·001
Glucokinase	0·928 $\pm$ 0·081	0·447 $\pm$ 0·059	1·44 $\pm$ 0·24	0·258 $\pm$ 0·133	< 0·001	0·06	0·001	N.S.	< 0·01
Glucose 6-phosphate dehydrogenase	1·12 $\pm$ 0·14	0·895 $\pm$ 0·097	3·22 $\pm$ 0·41	1·46 $\pm$ 0·08	N.S.	< 0·001	0·05	0·001	< 0·001
6-Phosphogluconate dehydrogenase	2·96 $\pm$ 0·07	2·44 $\pm$ 0·14	3·65 $\pm$ 0·32	2·57 $\pm$ 0·11	< 0·01	0·05	< 0·01	N.S.	< 0·01
Transketolase	1·67 $\pm$ 0·08	2·01 $\pm$ 0·16	1·83 $\pm$ 0·07	1·65 $\pm$ 0·08	0·08	N.S.	N.S.	N.S.	N.S.
Transaldolase	1·38 $\pm$ 0·04	1·61 $\pm$ 0·06	1·73 $\pm$ 0·06	1·61 $\pm$ 0·05	< 0·01	< 0·001	< 0·01	N.S.	N.S.
Ribose 5-phosphate isomerase	3·10 $\pm$ 0·08	2·94 $\pm$ 0·13	4·07 $\pm$ 0·10	3·32 $\pm$ 0·10	N.S.	< 0·01	N.S.	0·05	< 0·001
Ribulose 5-phosphate epimerase	10·82 $\pm$ 0·49	9·28 $\pm$ 0·43	11·38 $\pm$ 0·50	10·17 $\pm$ 0·43	< 0·05	N.S.	N.S.	N.S.	< 0·01
Phosphoglucose isomerase	44·5 $\pm$ 3·7	46·3 $\pm$ 1·2	51·8 $\pm$ 4·6	60·0 $\pm$ 9·3	N.S.	N.S.	N.S.	N.S.	N.S.



Fisher's *P*				
Control versus starved	0·048	<0·001	0·011	<0·001
Control versus high-carbohydrate diet	N.S.	N.S.	0·016	N.S.
Control versus high-fat diet	N.S.	<0·001	N.S.	0·002
Starved versus high-carbohydrate diet	0·034	<0·001	<0·001	0·002
Starved versus high-fat diet	0·007	N.S.	<0·001	0·001



Fisher's *P*					
Control versus starved	<0·001	<0·001	<0·001	<0·001	<0·001
Control versus high-carbohydrate diet	N.S.	N.S.	N.S.	N.S.	N.S.
Control versus high-fat diet	N.S.	N.S.	N.S.	N.S.	N.S.
Starved versus high-carbohydrate diet	<0·001	0·002	0·011	0·004	<0·001
Starved versus high-fat diet	<0·001	<0·001	0·009	<0·001	0·005

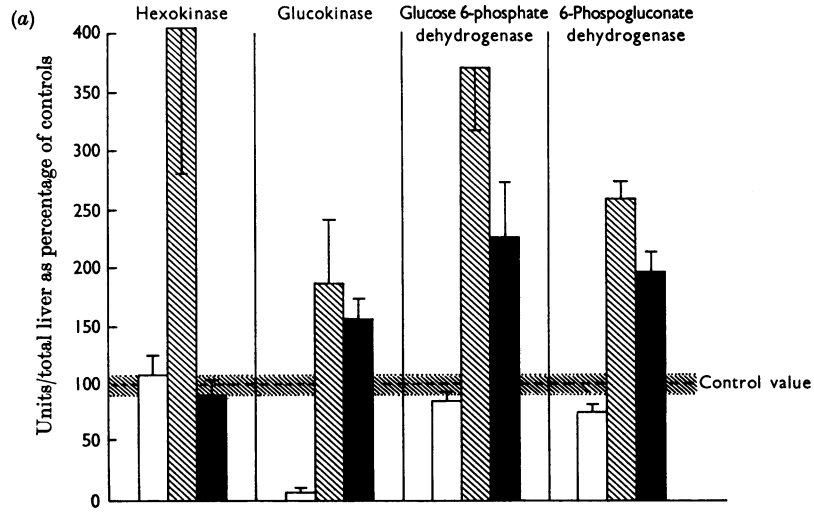
Fig. 1. Activities of enzymes of the pentose phosphate pathway and of hexokinase and glucokinase in liver of normal rats, starved rats and starved rats re-fed with a high-carbohydrate or high-fat diet. All results are expressed as units/total liver and are given as percentages of the appropriate control value. The control value is shown by the horizontal broken line; the S.E.M. is given by the shaded area (mean ± S.E.M. for eight rats). The vertical columns are respectively: □, rats starved for 2 days (six rats); ▩, rats starved for 2 days and re-fed with a high-carbohydrate diet for 3 days (six rats); ■, rats starved for 2 days and re-fed with a high-fat diet for 3 days (six rats). The vertical lines give the S.E.M. values, for clarity shown in one direction only. Fisher's *P* values for comparison among the different groups are given at the foot of each column.

and 'overshoot' in the activity of glucose 6-phosphate dehydrogenase, a tendency also shown by glucokinase; the activities of hexokinase and all the enzymes of the pentose phosphate pathway were restored to within the range of normal values. Comparison of changes after re-feeding with high-fat diets with those after re-feeding with high-carbohydrate diets shows that almost identical restoration of activity was obtained by the non-oxidative enzymes of the pentose phosphate cycle, i.e. ribose 5-phosphate isomerase, ribulose 5-phosphate 3-epimerase, transketolase and transaldolase, in contrast with the well-known effects of these diets on the oxidative reactions of this cycle. Thus glucose 6-phosphate dehydrogenase activity was restored only to the control value when high-fat

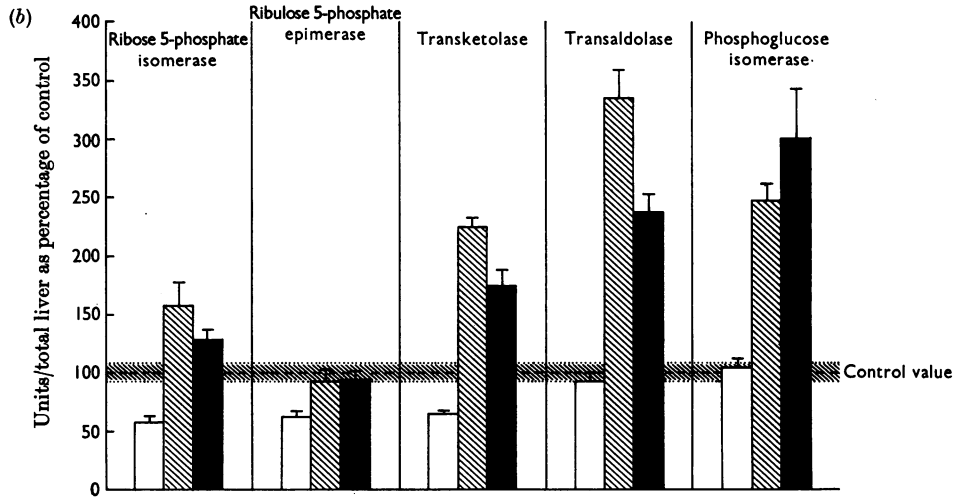
Table 2. *Effects of alloxan-diabetes and insulin treatment on the activities of the pentose phosphate cycle in rat liver*

A unit of enzyme activity is the amount catalysing the formation of 1 μmole of product/min. at 25°. Values are given as means ± S.E.M. Fisher's P values are given: where P is greater than 0·1 the value is quoted as N.S. (not significant). The alloxan-diabetic rats were used 3 weeks after administration of alloxan, and the insulin-treated rats received daily subcutaneous injections each of 2 units of protamine–zinc–insulin for 3 or 7 days.

Group no.	Control group 1	Diabetic group 2	Diabetic group treated with insulin (3 days) 3	Diabetic group treated with insulin (7 days) 4	Fisher's P values for comparison of groups			
					1 versus 2	2 versus 3	2 versus 4	1 versus 4
No. of animals	9	6	6	6				
Body wt. (g.)	199 ± 10	171 ± 9	205 ± 5	199 ± 8				
Liver wt. (g.)	9·21 ± 0·41	8·15 ± 0·52	14·56 ± 1·10	14·57 ± 1·46				
Protein content (mg./g.)	93 ± 2	111 ± 4	88 ± 2	95 ± 2				
Blood sugar (mg./100 ml.)	105 ± 5	473 ± 44	299 ± 58	389 ± 45				
Units/g. of liver								
Hexokinase	0·305 ± 0·054	0·383 ± 0·050	0·815 ± 0·228	0·189 ± 0·030	N.S.	0·09	< 0·01	0·08
Glucokinase	0·930 ± 0·124	0·064 ± 0·024	1·25 ± 0·41	1·01 ± 0·11	< 0·001	0·01	< 0·001	N.S.
Glucose 6-phosphate dehydrogenase	1·41 ± 0·06	1·37 ± 0·08	3·36 ± 0·29	2·07 ± 0·30	N.S.	< 0·001	< 0·05	< 0·05
6-Phosphogluconate dehydrogenase	2·65 ± 0·09	2·32 ± 0·11	4·53 ± 0·35	3·44 ± 0·27	< 0·05	< 0·001	< 0·01	0·01
Transketolase	1·49 ± 0·04	1·16 ± 0·06	2·25 ± 0·10	1·73 ± 0·05	0·001	< 0·001	< 0·001	< 0·01
Transaldolase	1·18 ± 0·06	1·28 ± 0·06	2·62 ± 0·14	1·50 ± 0·06	N.S.	< 0·001	< 0·05	< 0·01
Ribose 5-phosphate isomerase	3·58 ± 0·18	2·46 ± 0·25	3·80 ± 0·50	2·94 ± 0·13	< 0·05	< 0·05	N.S.	< 0·05
Ribulose 5-phosphate epimerase	11·74 ± 1·14	8·42 ± 0·57	6·96 ± 0·50	7·40 ± 0·54	< 0·05	N.S.	N.S.	< 0·01
Phosphoglucose isomerase	40·4 ± 2·8	51·1 ± 1·1	66·2 ± 2·3	81·4 ± 11·6	< 0·01	< 0·001	< 0·01	< 0·01



Fisher's P				
Control versus diabetic	N.S.	<0·001	N.S.	0·02
Diabetic versus diabetic+insulin (3 days)	0·04	<0·001	<0·001	<0·001
Diabetic versus diabetic+insulin (7 days)	N.S.	<0·001	0·016	<0·001
Control versus diabetic+insulin (7 days)	N.S.	0·05	0·002	<0·001



Fisher's P					
Control versus diabetic	<0·001	0·002	<0·001	N.S.	N.S.
Diabetic versus diabetic+insulin (3 days)	<0·001	0·026	<0·001	<0·001	<0·001
Diabetic versus diabetic+insulin (7 days)	<0·001	0·003	<0·001	<0·001	<0·001
Control versus diabetic+insulin (7 days)	0·069	N.S.	<0·001	<0·001	<0·001

Fig. 2. Activities of enzymes of the pentose phosphate pathway and of hexokinase and glucokinase in liver of alloxan-diabetic rats and alloxan-diabetic rats treated with insulin. All results are expressed as units/total liver and are given as percentages of the appropriate control value. The control value is shown by the horizontal broken line; the S.E.M. is given by the shaded area (mean ± S.E.M. for nine rats). The vertical columns are respectively: □, alloxan-diabetic rats (six rats); ■, alloxan-diabetic rats treated with 2 units of protamine–zinc–insulin for 3 days (six rats); ■, alloxan-diabetic rats treated with 2 units of protamine–zinc–insulin for 7 days (six rats). The vertical lines give the S.E.M. values, for clarity shown in one direction only (vertical downwards in two cases). Fisher's P values for comparison among the different groups are given at the foot of each column.

diets were given, and 6-phosphogluconate dehydrogenase activity failed to reach the control value and was significantly lower both in terms of units/g. of tissue and total activity in the whole liver. In addition, the well-documented effect of high-fat diet in failing to restore the glucokinase activity was observed here.

It thus appeared that the non-oxidative enzymes of the pentose phosphate pathway behaved similarly in response to these dietary modifications and were not sensitive to the nature of the food given, whether high-carbohydrate or high-fat. The complete restoration of transketolase and transaldolase by re-feeding with a high-carbohydrate diet after starvation for 72hr., as opposed to 48hr., was also observed.

The changes in glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities are in line with earlier work on the effect of starvation and re-feeding on the activities of these enzymes in liver (Glock & McLean, 1955; Tepperman & Tepperman, 1958, 1963; Fitch & Chaikoff, 1960; Potter & Ono, 1961; Niemeyer, Clark-Turri, Grarcés & Vergara, 1962; Johnson & Sassoon, 1967). Further, Benevenga *et al.* (1964) showed that the activities of the pentose phosphate-pathway metabolizing enzymes increased during a 4-day period of starvation when considered as activity/g. of liver.

Effect of alloxan-diabetes, with and without insulin treatment and glucagon treatment. The most marked change found in the alloxan-diabetic rats was a decrease in glucokinase activity to less than 10% of the control activity (Table 2). No change was found in hexokinase activity, an effect that is already well established (Viñuela, Salas & Sols, 1963; Salas, Viñuela & Sols, 1963; Sharma *et al.* 1963; Walker & Rao, 1964; Blumenthal, Abraham & Chaikoff, 1964; Sols, Sillero & Salas, 1965; Niemeyer, Pérez & Codaceo, 1967). None of the pentose phosphate-pathway enzymes even approached this magnitude of change; in fact, glucose 6-phosphate dehydrogenase, transaldolase and phosphoglucose isomerase activities were all within the normal range, 6-phosphogluconate dehydrogenase activity decreased by approx. 30% and the activities of the remaining enzymes of the cycle, ribose 5-phosphate isomerase, ribulose 5-phosphate epimerase and transketolase, all fell by about the same amount to approx. 60% of the control values (Table 2 and Fig. 2). Benevenga *et al.* (1964) reported a decrease of 30% in the overall activity of the pentose phosphate-pathway metabolizing enzymes in diabetes. More striking changes were found when alloxan-diabetic rats were treated with protamine–zinc–insulin for 3 days; here there was a great 'overshoot' in activity of all the enzymes studied with the exception of ribulose 5-phosphate epimerase. Of particular interest was the rise in hexokinase activity to about three to four times the normal activity, showing that under certain conditions liver hexokinase can be influenced by hormonal factors and that adaptations to glucose phosphorylation in liver may not necessarily be limited to glucokinase (see Ilyin, 1964; Sharma, Manjeshwar & Weinhouse, 1964; Walker & Rao, 1964). It could be that the combination of high blood sugar concentration and available insulin is responsible for the increase in hexokinase at this time. This increase in hexokinase actually brings the enzyme activity into the range of glucokinase activity in terms of units/g. of tissue (see Table 2). If the protamine–zinc–insulin treatment was continued for 7 days the hexokinase activity returned to the normal control value.

Although the activities of glucose 6-phosphate dehydrogenase, transketolase and transaldolase in terms of units/g. of liver are all closely similar in the control animals and alloxan-diabetic group, the more marked increase in glucose 6-phosphate dehydrogenase activity in alloxan-diabetic rats treated with protamine–zinc–insulin for 3 days results in an apparent shift in the rate-limiting step to transketolase (see Table 2). It is also noteworthy that ribulose 5-phosphate epimerase, the most active enzyme of the pentose phosphate cycle, shows the least change in these groups of experimental animals. The 'overshoot' effect of protamine–zinc–insulin treatment on the enzyme activities is less marked when the rats have been treated for 7 days; particularly is this true for hexokinase and glucose 6-phosphate dehydrogenase.

Neither the pattern nor the magnitude of the changes produced by glucagon treatment matched those found in the alloxan-diabetic groups (see Table 3), although there is some reason to consider these hormonal conditions together since in both cases there is a high glucagon content relative to insulin. In terms of units/g. of tissue, transaldolase, ribose 5-phosphate isomerase and ribulose 5-phosphate epimerase activities were all significantly lower. However, this treatment resulted in an increased liver weight and, when considered on this basis, the only significant difference was a rise in glucokinase activity (Table 3).

Effect of adrenalectomy and treatment with cortisone. These results are summarized in Table 4. In adrenalectomized rats the two most important changes were the rise in hexokinase activity and the fall in transketolase activity. Ribulose 5-phosphate epimerase and phosphoglucose isomerase activities were also decreased. The increase in hexokinase activity again points to hormonal control being exerted on this enzyme and not solely on glucokinase, in agreement with the work of Ilyin (1964). Sharma *et al.* (1964) have also reported values

Table 3. *Effect of treatment with glucagon on the activities of enzymes of the pentose phosphate pathway in rat liver*

A unit of enzyme activity is the amount catalysing the formation of 1 μmole of product/min. at 25°. The results are given as means ± S.E.M. Fisher's P values are given; where P is greater than 0·1 the value is quoted as N.S. (not significant). The glucagon-treated rats received subcutaneous injections of 100 μg. of glucagon on each of 3 successive days.

	Control	Glucagon-treated	Fisher's P
No. of animals	8	6	
Body wt. (g.)	184±6	165±4	
Liver wt. (g.)	8·46±0·41	9·69±0·48	
Protein content (mg./g.)	92±3	86±2	
Units/g. of liver			
Hexokinase	0·246±0·049	0·195±0·008	N.S.
Glucokinase	0·930±0·080	1·11±0·08	N.S.
Glucose 6-phosphate dehydrogenase	1·12±0·14	0·86±0·04	N.S.
6-Phosphogluconate dehydrogenase	2·95±0·06	2·96±0·21	N.S.
Transketolase	1·67±0·08	1·54±0·06	N.S.
Transaldolase	1·38±0·04	1·17±0·03	<0·01
Ribose 5-phosphate isomerase	3·10±0·08	2·68±0·09	<0·01
Ribulose 5-phosphate epimerase	10·82±0·49	9·79±0·27	<0·01
Phosphoglucose isomerase	44·4±3·7	38·2±1·8	N.S.
Units/total liver			
Hexokinase	2·19±0·57	1·86±0·09	N.S.
Glucokinase	7·76±0·70	10·73±0·97	<0·05
Glucose 6-phosphate dehydrogenase	9·77±1·72	8·40±0·74	N.S.
6-Phosphogluconate dehydrogenase	25·0±1·2	29·0±2·9	N.S.
Transketolase	13·9±0·48	14·9±0·88	N.S.
Transaldolase	11·7±0·89	11·3±0·66	N.S.
Ribose 5-phosphate isomerase	26·3±1·8	25·7±1·9	N.S.
Ribulose 5-phosphate epimerase	92±5	95±6	N.S.
Phosphoglucose isomerase	372±20	373±32	N.S.

suggesting changes in adrenalectomized animals (1·30 ± 0·32 and 2·53 ± 0·08 for control and adrenalectomized groups respectively, three rats only in each group). It is noteworthy that in adrenalectomized rats transketolase is again an enzyme under hormonal control, and this once more points to the key role of this enzyme in controlling the non-oxidative reactions of the cycle. Treatment of adrenalectomized rats with cortisone for 3 days largely reversed these changes, and no significant differences were then found between hexokinase and transketolase activities of the control and experimental groups; however, glucokinase activity was significantly decreased by this treatment (Table 4). Treatment of normal animals with high doses of cortisone (5 mg./day for 3 days) failed to produce any response in the activities of hexokinase, glucokinase or the enzymes of the pentose phosphate pathway.

As shown in Table 4 no significant changes were found in glucose 6-phosphate dehydrogenase or 6-phosphogluconate dehydrogenase activity, in contrast with the results of Huggins & Yao (1959), who found both enzyme activities decreased, the former more than the latter, and Willmer (1960), who found that glucose 6-phosphate dehydrogenase activity was decreased in adrenalectomized animals. It is possible that these differences may be accounted for by the paired-feeding technique used in the present experiments. Both dehydrogenases of the pentose phosphate pathway are markedly affected by food intake, and it is important in adrenalectomy, where the food intake decreases, that the controls should be carefully matched.

Benevenga *et al.* (1964) have reported that the overall activity of the pentose phosphate-pathway metabolizing enzymes fell to 62% of the control activity, and the present observations show that this may be largely ascribed to a decrease in transketolase activity. Cortisone treatment of normal rats failed to increase the activity of any of the enzymes of the pentose phosphate pathway significantly, contrasting with the raised overall activity reported by Benevenga *et al.* (1964).

Effects of thyroidectomy, hypophysectomy and treatment of hypophysectomized rats with thyroxine and growth hormone. The results of these experiments are shown in Tables 5 and 6. It was found that in hypothyroidism both glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities were decreased, the former more markedly, in agreement with the results of Huggins & Yao (1959). These results are also in general agreement with the increased activity of the

Table 4. *Effects of adrenalectomy and treatment of adrenalectomized rats with cortisone on the activities of enzymes of the pentose phosphate cycle in rat liver*

A unit of enzyme activity is the amount catalysing the formation of 1 μmole of product/min. at 25°. Values are given as means ± S.E.M. Fisher's *P* values were calculated for corresponding pairs of animals because of the paired-feeding technique used; where *P* is greater than 0·1 the value is quoted as N.S. (not significant). The rats were used 7 days after adrenalectomy; a group of adrenalectomized rats were treated with cortisone (100 μg./day) for 3 days subcutaneously.

	Control	Adrenalectomized	Fisher's *P*
No. of animals	6	6	
Body wt. (g.)	165 ± 4	164 ± 5	
Liver wt. (g.)	7·74 ± 0·40	7·30 ± 0·43	
Protein content (mg./g.)	96 ± 1·0	91 ± 1·5	
Units/g. of liver			
Hexokinase	0·290 ± 0·03	0·483 ± 0·06	< 0·05
Glucokinase	1·10 ± 0·06	1·06 ± 0·16	N.S.
Glucose 6-phosphate dehydrogenase	1·30 ± 0·09	1·53 ± 0·14	N.S.
6-Phosphogluconate dehydrogenase	3·04 ± 0·14	2·84 ± 0·03	N.S.
Transketolase	1·31 ± 0·03	1·09 ± 0·05	< 0·01
Transaldolase	1·63 ± 0·06	1·54 ± 0·02	N.S.
Ribose 5-phosphate isomerase	3·84 ± 0·22	3·89 ± 0·15	N.S.
Ribulose 5-phosphate epimerase	13·8 ± 0·4	11·8 ± 0·2	0·01
Phosphoglucose isomerase	58·5 ± 2·1	51·5 ± 1·8	< 0·05

	Control	Adrenalectomized treated with cortisone	Fisher's *P*
No. of animals	4	4	
Body wt. (g.)	170 ± 4	168 ± 3	
Liver wt. (g.)	8·44 ± 0·61	7·63 ± 0·27	
Protein content (mg./g.)	90 ± 3	88 ± 1	
Units/g. of liver			
Hexokinase	0·352 ± 0·032	0·323 ± 0·047	N.S.
Glucokinase	1·09 ± 0·11	0·83 ± 0·06	< 0·05
Glucose 6-phosphate dehydrogenase	1·44 ± 0·05	1·26 ± 0·14	N.S.
6-Phosphogluconate dehydrogenase	2·89 ± 0·05	2·75 ± 0·19	N.S.
Transketolase	1·86 ± 0·05	1·83 ± 0·05	N.S.
Transaldolase	1·25 ± 0·05	1·39 ± 0·04	0·05
Ribose 5-phosphate isomerase	3·70 ± 0·12	3·79 ± 0·11	N.S.
Ribulose 5-phosphate epimerase	10·0 ± 0·3	9·7 ± 0·3	N.S.
Phosphoglucose isomerase	50·0 ± 1·5	44·6 ± 1·3	0·05

Table 5. *Effects of thyroidectomy on the activities of enzymes of the pentose phosphate cycle in rat liver*

A unit of enzyme activity is the amount catalysing the formation of 1 μmole of product/min. at 25°. Values are given as means ± S.E.M. Fisher's *P* values are given; where *P* is greater than 0·1 the value is quoted as N.S. (not significant). The animals were killed on the eleventh day after thyroidectomy.

	Control	Thyroidectomized	Fisher's *P*
No. of animals	6	6	
Body wt. (g.)	156 ± 5	152 ± 5	
Liver wt. (g.)	6·06 ± 0·26	6·01 ± 0·22	
Protein (mg./g.)	101 ± 4	100 ± 3	
Units/g. of liver			
Glucose 6-phosphate dehydrogenase	1·50 ± 0·05	0·87 ± 0·06	< 0·001
6-Phosphogluconate dehydrogenase	2·90 ± 0·16	2·18 ± 0·06	< 0·001
Transketolase	1·95 ± 0·09	1·32 ± 0·02	< 0·01
Transaldolase	1·27 ± 0·08	1·24 ± 0·05	N.S.
Ribose 5-phosphate isomerase	4·81 ± 0·04	3·26 ± 0·30	< 0·01

oxidative enzymes of the pentose phosphate cycle shown in hyperthyroidism (Glock & McLean, 1955; Huggins & Yao, 1959).

Of the non-oxidative enzymes of the cycle both ribose 5-phosphate isomerase and transketolase were lower in activity in thyroidectomized animals,

Table 6. *Effects of hypophysectomy and treatment with thyroxine and growth hormone on transketolase activity of rat liver*

A unit of enzyme activity is defined as the amount catalysing the formation of 1 μmole of product/min. at 25°. Values are given as means ± S.E.M. Fisher's P values are given for comparison of each group with the control rats; where P is greater than 0·1 this is quoted as N.S. (not significant). The hypophysectomized rats were treated with 0·5 mg. of growth hormone or 10 μg. of L-thyroxine/day for 8 days. The food intake was limited to that of the hypophysectomized group.

	Control	Hypophysectomized	Hypophysectomized + growth hormone	Hypophysectomized + thyroxine	Fisher's P		
Group no. ...	1	2	3	4	1 versus 2	1 versus 3	1 versus 4
No. of animals	6	6	4	10			
Body wt. (g.)	150 ± 7	131 ± 10	160 ± 7	140 ± 4			
Liver wt. (g.)	7·27 ± 0·43	5·06 ± 0·14	5·71 ± 0·27	5·10 ± 0·34			
Units/g. of liver	0·72 ± 0·08	0·54 ± 0·02	0·73 ± 0·11	0·69 ± 0·04	0·05	N.S.	N.S.
Units/total liver	5·24 ± 0·065	2·70 ± 0·13	4·19 ± 0·31	3·54 ± 0·24	< 0·01	N.S.	< 0·05

whereas in contrast with this transaldolase did not change significantly (Table 5).

The effect of pituitary hormones on transketolase was also investigated. It was found that hypophysectomy caused a 50% fall in the activity of this enzyme that was partially reversed by treatment with thyroxine for 8 days. Although the increase produced by thyroxine treatment was significant, it by no means completely restored the transketolase activity and greater restoration was found in growth-hormone-treated rats, where the transketolase reached 80% of the control activity after treatment with this hormone for 1 week (Table 6). Hypophysectomy has been shown to cause marked decreases in both glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities, and a differential effect of thyroxine on the restoration of these enzyme activities has been shown. The former enzyme responded less markedly than the latter, which was completely restored to normal activity by thyroxine treatment (Huggins & Yao, 1959). These results again point to the requirement of more than one pituitary hormone in the maintenance and function of the pentose phosphate pathway.

DISCUSSION

The main purpose of the present experiments was to determine if control of the non-oxidative reaction of the cycle occurred at a specific enzyme or if all the enzymes of the group behaved as a composite whole, i.e. as a constant-proportion group of enzymes.

Measurements of the relative activities of the enzymes of the non-oxidative part of the pentose cycle show that transketolase and transaldolase have approximately equal activities and are the lowest in the group, and thus either of these enzymes could be a control point in the non-oxidative reactions of the cycle.

Transketolase may also be considered to be at a branch point in metabolism since ribose 5-phosphate may be converted into either phosphoribosyl pyrophosphate or sedoheptulose 7-phosphate by reaction with ATP or xylulose 5-phosphate respectively, the second reaction being catalysed by transketolase. Another reaction in which transketolase and transaldolase may perhaps both be considered to act at a branch point of metabolism is at fructose 6-phosphate in the reversal of non-oxidative reactions of the pentose phosphate cycle (Bonsignore, Pontremoli, Mangiarotti, DeFlora & Mangiarotti, 1962). Thus transketolase and to a smaller extent transaldolase meet several of the requirements held to be important in controlling reactions of metabolic pathways.

The present experiments show two patterns of response to hormonal control in rat liver. First, where very large changes occur in glucose metabolism such as in alloxan-diabetes and in insulin-treated alloxan-diabetic rats, the whole complex of the non-oxidative enzymes is modified. Starvation and re-feeding with a high-carbohydrate diet is another such case. Where milder changes occur in glucose metabolism and only certain of the enzyme activities are changing, the more specific effect on transketolase is seen and it is generally found that this enzyme changes in activity when others of the cycle are relatively unaffected as, for example, in adrenalectomized rats (Table 4); in thyroidectomized rats there is a significant decrease in transketolase activity with no apparent alteration in transaldolase activity (Table 5).

A difference between the response of the glucose 6-phosphate dehydrogenase and non-oxidative enzymes of the pentose phosphate cycle is clearly

seen in the starvation–re-feeding experiments. The response of the former enzyme to the high-carbohydrate diet was a massive increase in activity, much less change being found as a result of the high-fat diet; in contrast with this, the activities of the non-oxidative reactions and 6-phosphogluconate dehydrogenase increased almost identically as a result of either diet, being restored to the control activity rather than showing the massive 'overshoot' characteristic of the response of glucose 6-phosphate dehydrogenase to high carbohydrate intake (Tepperman & Tepperman, 1958, 1963; Fitch & Chaikoff, 1960; Potter & Ono, 1961). Tepperman & Tepperman (1963) have discussed the possible signals triggering the response of glucose 6-phosphate dehydrogenase, but no evidence could be found for a relationship with either the glucose 6-phosphate content of the liver or the rate of reoxidation of NADPH. The response of the first enzyme of the pentose phosphate pathway thus appears to be unique in the cycle.

In the insulin-treated alloxan-diabetic animals all the enzyme activities with the exception of the highly active ribulose 5-phosphate epimerase rose to supranormal values, a pattern of change found also in studies of the effect of insulin on the activities of urea-cycle enzymes in liver of alloxan-diabetic rats (McLean & Novello, 1965). Thus in different hormonal and dietary conditions the response of the pentose phosphate pathway varies, no one pattern prevailing.

In considering the many reports on control of glucose phosphorylation in liver, most emphasis in the literature has been put on glucokinase; indeed, the changes in the activity of this enzyme are outstanding in both alloxan-diabetes and during starvation and re-feeding. Measurements of glucokinase and hexokinase activities in the present experiments did, however, point to the possibility that hexokinase might be a target for hormone action. The conditions found to cause changes in hexokinase activity were adrenalectomy and insulin treatment; in addition, re-feeding after a period of starvation also caused a rise in hexokinase activity.

Interesting hormone-induced changes in hexokinase activity have been found in studies with rabbit liver (Formina, 1962; Ilyin, 1964). Here alterations were found in the hexokinase activity in both the soluble plus microsomal fractions and in the mitochondrial fraction under different hormonal conditions. Rabbit liver, in contrast with rat liver, contains a large proportion of mitochondria-bound hexokinase. Cortisone treatment of rabbits caused decreases in the soluble and mitochondrial hexokinase activities, whereas in alloxan-diabetic rabbits the most striking changes were found in the soluble fraction of the liver, where a marked decrease in hexokinase activity was found.

Growth hormone and adrenal glucocorticoids were identified as 'physiological insulin antagonists' many years ago, and it is known that hypophysectomy and adrenalectomy correct some defects in glucose metabolism in alloxan-diabetic rats (see Kipnis & Stein, 1964). The sensitivity of liver hexokinase to these hormones is of interest in this context. The presence of bound forms of hexokinase and changes in this fraction under different hormonal conditions clearly indicate a further form of metabolic control (Walters & McLean, 1968*a*,*b*).

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